Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells.

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Authors: Daniel Ramskold, Shujun Luo, Yu-Chieh Wang, Robin Li, Qiaolin Deng, Omid R Faridani, Gregory A Daniels, Irina Khrebtukova, Jeanne F Loring, Louise C Laurent, Gary P Schroth, Rickard Sandberg

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Public Summary:
For the first time, we captured single circulating tumor cells from the blood of cancer patients, and identified all of the genes that are active in these cells. These cells may be involved in seeding metastases that take root in other parts of the body. We developed two new methods to accomplish the single cell sequencing: a novel cell-capture system to isolate rare circulating tumor cells from blood, and a new method ("Smart-Seq") for analyzing messenger RNA in single cells by next-generation sequencing technology. We found that the circulating melanoma tumor cells express genes that are associated with normal pigment cells (melanocytes) and with cultured cells derived from melanoma tumors. For this particular example, we are able to identify new biomarkers for circulating tumor cells that may help us identify and target cancer stem cells. For the broader area of stem cell research, the single cell sequencing technique will be valuable for examining individual cells in culture populations; for example, in unpublished work, we have analyzed multiple individual cells plucked out of a culture of hepatocyte-like cells derived from human pluripotent stem cells. By examining the diversity of gene expression profiles among individual cells in a culture, we can determine the identities of the cells, including those that are contaminants with inappropriate phenotypes (cardiac cells in a hepatocyte culture, for example). This technique can also be used to identify the types of neurons that develop from disease-specific iPSCs, comparing them to non-diseased samples. Since many developmental neurological disorders are characterized by an overabundance or a dearth of a particular type of neuron, we can use the single cell technique as a means to characterize "disease in a dish" experiments.

Scientific Abstract:
Genome-wide transcriptome analyses are routinely used to monitor tissue-, disease- and cell type-specific gene expression, but it has been technically challenging to generate expression profiles from single cells. Here we describe a robust mRNA-Seq protocol (Smart-Seq) that is applicable down to single cell levels. Compared with existing methods, Smart-Seq has improved read coverage across transcripts, which enhances detailed analyses of alternative transcript isoforms and identification of single-nucleotide polymorphisms. We determined the sensitivity and quantitative accuracy of Smart-Seq for single-cell transcriptomics by evaluating it on total RNA dilution series. We found that although gene expression estimates from single cells have increased noise, hundreds of differentially expressed genes could be identified using few cells per cell type. Applying Smart-Seq to circulating tumor cells from melanomas, we identified distinct gene expression patterns, including candidate biomarkers for melanoma circulating tumor cells. Our protocol will be useful for addressing fundamental biological problems requiring genome-wide transcriptome profiling in rare cells.